Effect of feeding different levels of dietary protein and addition of baker's yeast (Saccharomyces cerevisiae) on Awassi lambs

performance. 3-Blood parameters

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Abstract

24 Awassi lambs were used to study biochemical changes in blood parameters as affected by concentrate diets containing different levels of dietary crude protein (CP) (low, medium and high) offered with or without baker's yeast (Saccharomyces cerevisiae, SC, (0 or 0.5%). Concentrate diets were offered to lambs at rate of 3% of live Body weight (BW) in addition to free choice of barley straw. Blood samples were withdrawn from lambs before feeding (0 time), 3 and 6 hrs post feeding. Results showed that blood urea nitrogen (BUN) concentrations were inversely affected by the mentioned factors, where, it increased with each increase in CP level, higher ($P<0.01$) value (45.73 mg.100 ml^{-1}) was detected with high level as compared to that detected with low level (40.97 mg.100 ml^{-1}), while, it decreased ($P<0.01$) from 44.88 to 41.01 mg/100 ml due to addition of yeast. Higher blood glucose (BG) ($P<0.01$) and blood total protein (BTP) ($P<0.05$) concentrations were detected in blood samples withdrawn from yeast supplemented lambs. Increasing level of dietary protein had no effect on BG, BTP and blood triglyceride (BTG). The concentration of blood components associated lipid { blood cholesterol (BCH) and BTG} were not affected by addition of SC.

Key words: Awassi lambs, dietary protein, baker's yeast and blood parameters

Introduction

Ruminant's diets must be formulated properly for crude protein (CP) and energy because these two nutrients have a substantial impact on production (29). However, the nutritional status of growing animals is of utmost importance, especially the status of protein nutrition of the animal.

Protein requirements are thought to be a function of many variables and not a specific figure for all conditions (22). Sheep are known for their excellent ability to utilize organic feed; however production effectiveness is generally lower in this species than in other farm animals. Therefore, attempts are made to supplement animal diets with feed additives stimulating productivity. One of such natural stimulator, with probiotic properties is Saccharomyces cerevisiae yeast which has a wide spectrum of activity (20).

On the other hand, blood plays an important role in carrying end products of metabolism processes out of body tissues and supplying these tissues with different nutrients; these nutrients differ in concentrations according to differences in diets and diet components through their interaction in metabolic pathways naturally occurring in different body tissues (2). Therefore, understanding changes naturally occurred in gastrointestinal tract can be enhanced by blood characterization. Huntington, et. al., (13) considered blood urea nitrogen as a function of CP intake. The relationship between dietary amino acid (AA) intakes and serum AA patterns in ruminants is obscured by the fermentative activity of the rumen, and the serum AA patterns are more closely related to the AA content of ruminal microorganisms and dietary protein escaping ruminal degradation (5).

Materials and Methods

24 Awassi male lambs weighing 26.5 kg and 4-6 months of age were used to investigate changes in rumen fermentation characteristics due to feeding three levels of dietary CP (11.5, 13.5 and 15.5%), each level was offered either alone or with the addition of baker's yeast{Saccharomyces cerevisiae, SC, (0 or 0.5%)}. Concentrate diets were formulated including these variables

and offered to lambs at rate of 3% of live BW in addition to free choice of barley straw. The commercial product of baker's yeast SC used as an additive and it contained about 5.6×10^8 colony forming unit (CFU).

Chemical analysis of diets including dry matter (DM), organic matter (OM), crude protein (CP), ether extracts (EE) and crude fiber (CF) were determined according to AOAC (4). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the method of Goering and Van Soest (10).

Blood samples (10 ml) were withdrawn from lambs via jugular venipuncture into vacutainer tubes, which were immediately placed in refrigerator, before morning feeding (zero time) and after0 3 and 6 hrs to determine blood glucose (BG), blood total protein (BTP), blood urea nitrogen (BUN), blood triglycerides (BTG) and blood cholesterol (BCH) concentrations. Blood samples were centrifuged and separated serum was collected and stored at -20 C^o until analysis was perfumed. BG was immediately measured using Accu-Chek electronic device as described by Al-Saady (3), Where, BG was read 5 second following putting the sample in the device. Other blood parameters were measured spectrophotometerically.

Data obtained was statistically analyzed using 3×2 factorial experiment design using completely randomized design model (CRD) procedure by (25). Sampling time was not taking into account as a factor affecting blood parameters to avoid complications. Duncan's multiple range tests was used to determine the significance of differences between treatments means (7). Formulation and chemical composition of concentrate diets, and straw are presented in tables 1, 2 and 3 respectively.

Table 1- The formulation of experimental concentrate diets (%)

Level of CP %	11.50		13.50		15.50	
SC %	0	0.5	0	0.5	0	0.5
Treatments no.	T_1	T_2	T_3	T_4	T_5	T_6
Ingredients %						
Barley	40	40	40	40	40	40
Wheat bran	35	35	35	35	35	35
Yellow corn	18	18	13	13	8	8
SBM	5	5	10	10	15	15
baker's yeast	0	0.5	0	0.5	0	0.5
Mineral and vitamin mixture	2	2	2	2	2	2

Table 2- Chemical analysis of ingredients used in formulating of concentrate diets.

Ingredients	Barley	Yellow corn	Soybean meal	Wheat bran
DM %	92.31	91.21	90.78	90.15
% of DM				
OM	90.32	92.60	91.23	92.00
CP	8.40	8.55	43.09	13.82
CF	6.24	3.89	5.31	9.60
EE	3.20	4.63	2.65	4.96
NFE	72.48	75.53	40.18	63.62
NDF	25.22	13.72	45.46	50.50
ADF	5.78	6.25	10.85	13.23
Cellulose	4.76	4.49	8.72	10.22
Hemicellulose	19.44	7.47	34.61	37.27
ADL	1.02	1.76	2.13	3.01

Table 3- Chemical composition of different concentrate diets and straw (% on DM basis).

Items/ diets	Concentrate diets						Barley straw
Level of CP %	11.50		13.50		15.50		
Addition of SC %	0	0.5	0	0.5	0	0.5	
DM	93.71	93.95	93.89	93.97	94.16	94.00	95.72
OM	93.47	93.72	93.97	94.49	93.62	94.18	90.19
CP	11.38	11.65	13.36	13.56	15.38	15.62	2.43
CF	7.00	6.93	7.00	6.72	6.85	6.75	40.17
EE	2.89	2.57	3.01	2.54	3.10	2.29	2.09
NFE	72.20	72.57	70.60	71.67	68.29	69.52	45.50
NDF	36.02	40.73	34.41	35.83	35.20	39.68	72.94
ADF	8.56	9.52	7.33	7.99	8.38	8.13	51.96
Cellulose	6.30	6.92	5.08	5.91	5.87	6.03	38.93

Hemicellulose	27.46	31.21	27.08	27.84	26.82	31.55	20.98
ADL	2.26	2.60	2.25	2.08	2.51	2.10	13.03
RDN	1.27	1.30	1.50	1.51	1.72	1.74	0.15
UDN	0.54	0.55	0.64	0.65	0.73	0.74	0.23
* ME	12.13	12.17	12.10	12.10	12.02	11.96	6.83

*Metabolizable energy (ME) values are estimated according to equation of Kearl (18).

ME (MJ/kg DM) = [- 0.45 + (0.04453×% TDN)] × 4.184

TDN is estimated according to the following equations

TDN for roughages (% of DM) = -17.2649+1.2120%CP +0.8352%NFE+2.4637% EE+0.4475 % CF

TDN for concentrate (% of DM) = 40.3227+0.5398 % CP+0.4448 % NFE+1.4218% EE–0.7007 % CF

Results and discussion

Determination of blood parameters aimed to investigate the biochemical changes may occur during an experiment due to nutritional circumstances. This is mainly because metabolic processes can be reflected on these changes. On this basis, Valkeners, et. al., (28) reported that diurnal variations in ruminal NH_3-N concentrations and blood urea concentrations were greatly influenced by the feeding patterns of the diet. Therefore, changes in some blood parameters may possibly help to explain the beneficial effect of additives in the diet (21).

The following discussion will deal with mean effect of the factors involved in a current study, including the level of dietary protein, addition of yeast and the interaction between them on the blood parameters including BG, BTP, BUN, BTG and BCH. Regarding the diurnal changes in these parameters in sampling time involved, illustration will be limited to figures to avoid unnecessary elaborateness.

The mean effect of level of protein on blood parameters during 24 hrs

The mean effect of levels of dietary CP on blood parameters during 24 hrs is presented in Table 4. Results revealed that increasing level of dietary CP had no significant effect on BG, BTP and BTG. Similar results concerning BG were observed by many researchers (21, 26). Whereas, Rusche, et.al. (23) demonstrated that increasing CP level resulted in increasing BG. Similar result concerning BTP was also reported by Shamoon et. al (26) due to increasing level of dietary CP in lambs. The little insignificant response of increasing dietary level of CP on BTP in a current study may be attributed to the fermentative activity of the rumen (5). Therefore, plasma amino acids (AA) patterns are more closely related to the fate of AA in the rumen.

Regarding blood triglyceride BTG, similar to our results, Salih (24) showed that there were no significant differences in BTG concentrations in samples withdrawn from lambs fed diets containing high level of CP, Whereas, a significant ($P<0.01$) differences were observed by Dosky (6) with Karadi ewes.

Results also revealed that increasing level of dietary CP led to a significant

(P<0.01) increase in BUN concentration, Similar result was reported by several investigations (12, 6). The increased of BUN concentrations can be largely explained by increased absorption of ruminal NH_3-N, resulting in greater quantities of NH_3-N being detoxified in the liver to form urea. James, et. al., (14) reported that urea is produced in the liver when ruminal degradation of dietary CP is not incorporated into microbial protein, but absorbed, causing an elevation of BUN.

Regarding BCH concentration, it was observed that increased dietary CP from low to medium level resulted in a significant (P<0.05) increase. Elevated cholesterol can be indicative of dietary lipid content or tissue catabolism (21). However, increasing dietary CP to high level resulted in a little insignificant decrease in BCH as compared with low level. Diurnal changes in blood parameters as affected by increasing levels of dietary CP were presented in Figures 1, 2, 3, 4 and 5.

Main effect of addition of SC (C) on blood parameters

The mean effect of addition of SC on blood parameters during 24 hrs is presented in Table 4. As shown BG concentration was significantly (P<0.01) increased due to addition of SC. Similar result was observed by Milewski and Sobiech (20). The increase (3.82 mg.100 ml^{-1}) in a current study can be simply explained by the propionogenesis process that proved to be improved by inclusion of yeast (17). Propionic acid is the main substrate for glucose synthesis in ruminants (11). However, Jouany, et. al., (16) reported that yeast metabolize the glucose and small oligosaccharides produced by amylolytic bacteria that adhere to starch grains and, as a consequence, less glucose is available for fermentative bacteria, the growth of which is then decreased; Saccharomyces cerevisiae cells can consume as much as 4g glucose per hrs per g DM.

Higher (P<0.05) BTP concentration was also noted due to addition of SC. This may be attributed to the release of protein substances stimulated by the presence of SC in addition to yeast matter per se. Similar finding was noticed by Galip, (9). Jouany, et. al., (15) observed that the ruminal non ammonia nitrogen (NAN) pools increased significantly (+30%) in

Table 4- Mean effect of level of dietary protein (A) on blood parameters during 24 hrs

Items	Level of dietary protein			Significance of effects
	Low	Medium	High	n = 72
BG mg.100 ml^{-1}	70.92 ± 0.66	69.45 ± 0.65	68.37 ± 0.59	NS
BTP g. 100 ml^{-1}	6.64 ± 0.12	6.81 ± 0.12	6.96 ± 0.10	NS
BUN mg.100 ml^{-1}	40.97^{b} ± 0.71	42.14^{b} ± 0.95	45.73^{a} ± 1.38	**
BTG mg.100 ml^{-1}	31.02 ± 0.78	32.23 ± 1.14	32.37 ± 0.78	NS
BCH mg.100 ml^{-1}	66.33^{ab} ± 1.40	71.11^{a} ± 1.62	64.71^{b} ± 2.25	*

Means in the same row with different superscripts are significantly different

* ($P<0.05$) ** ($P<0.01$) NS= Non significant

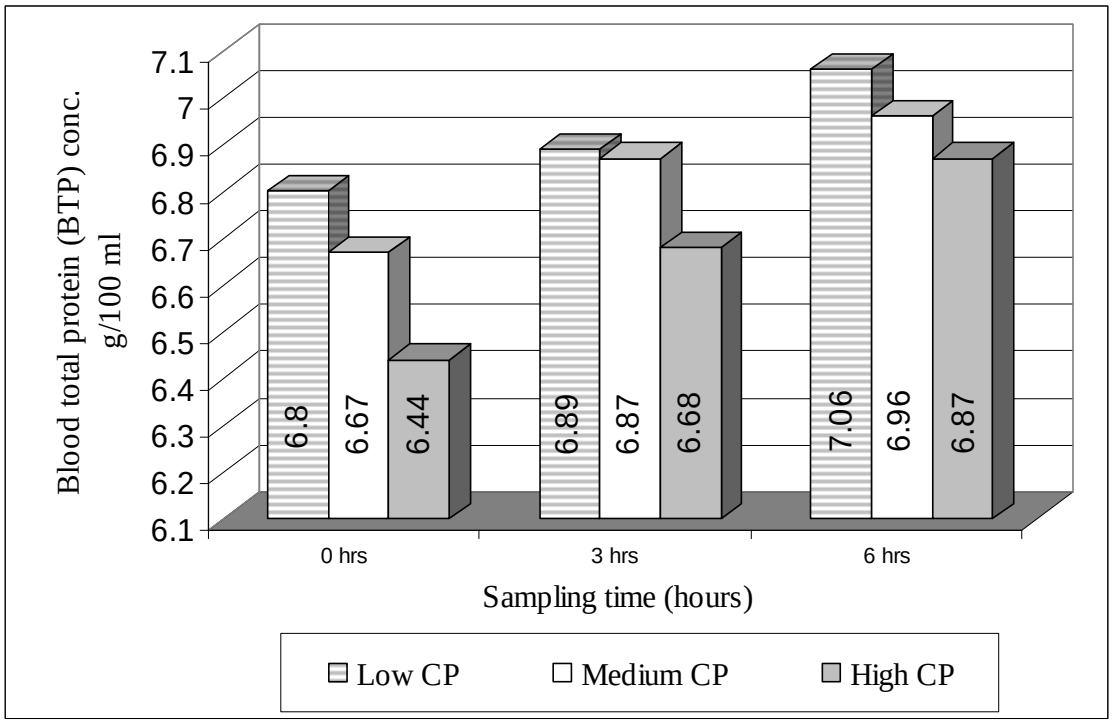

Figure 2- Diurnal pattern of blood total protein concentration (BTP) g/100 ml

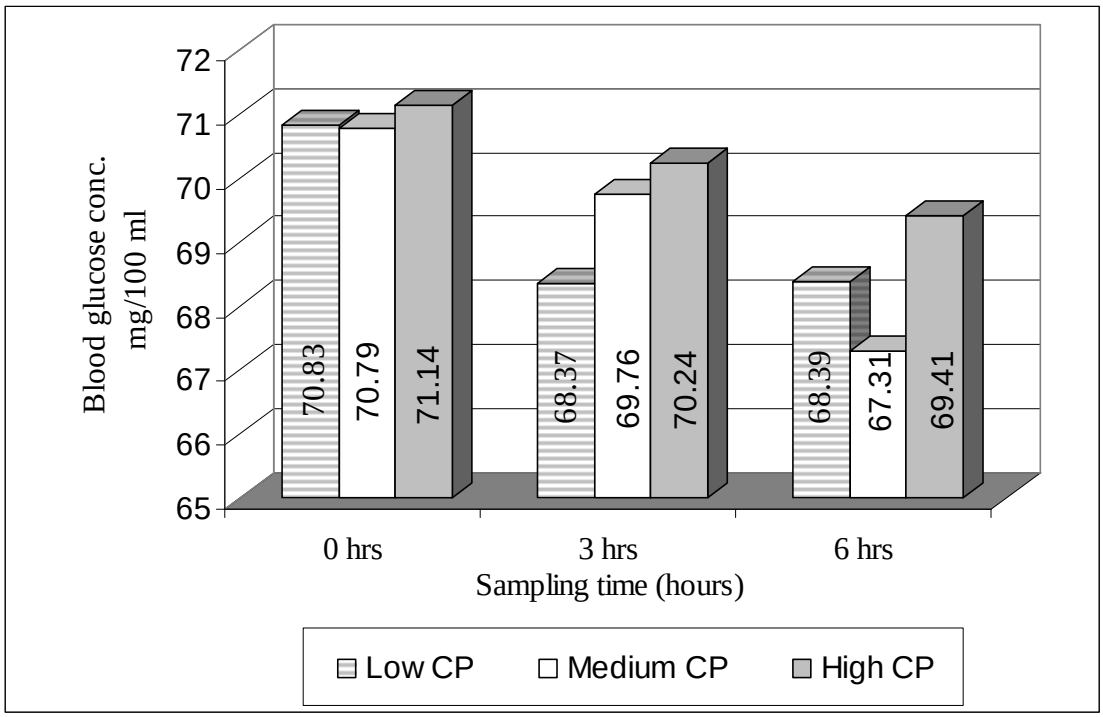

Figure 1- Diurnal pattern of blood glucose concentration (BG) mg/100 ml

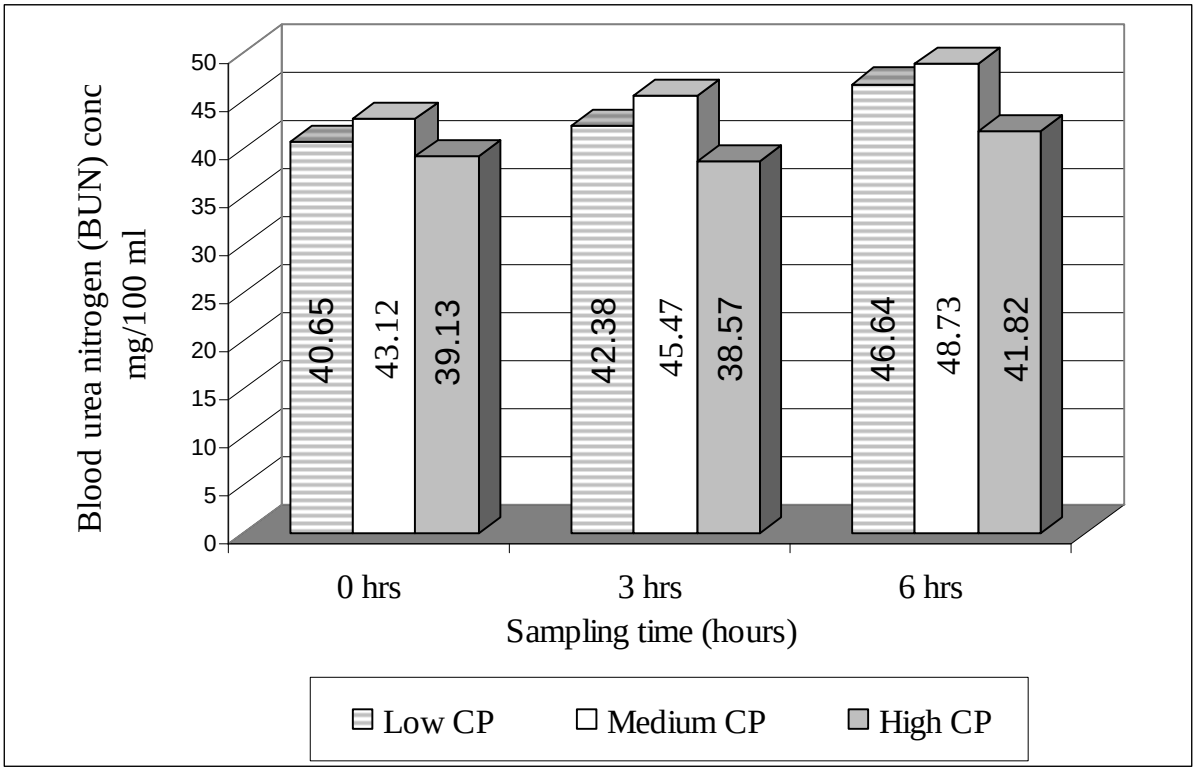

Figure 3- Diurnal pattern of blood urea nitrogen concentration (BUN) mg/100 ml

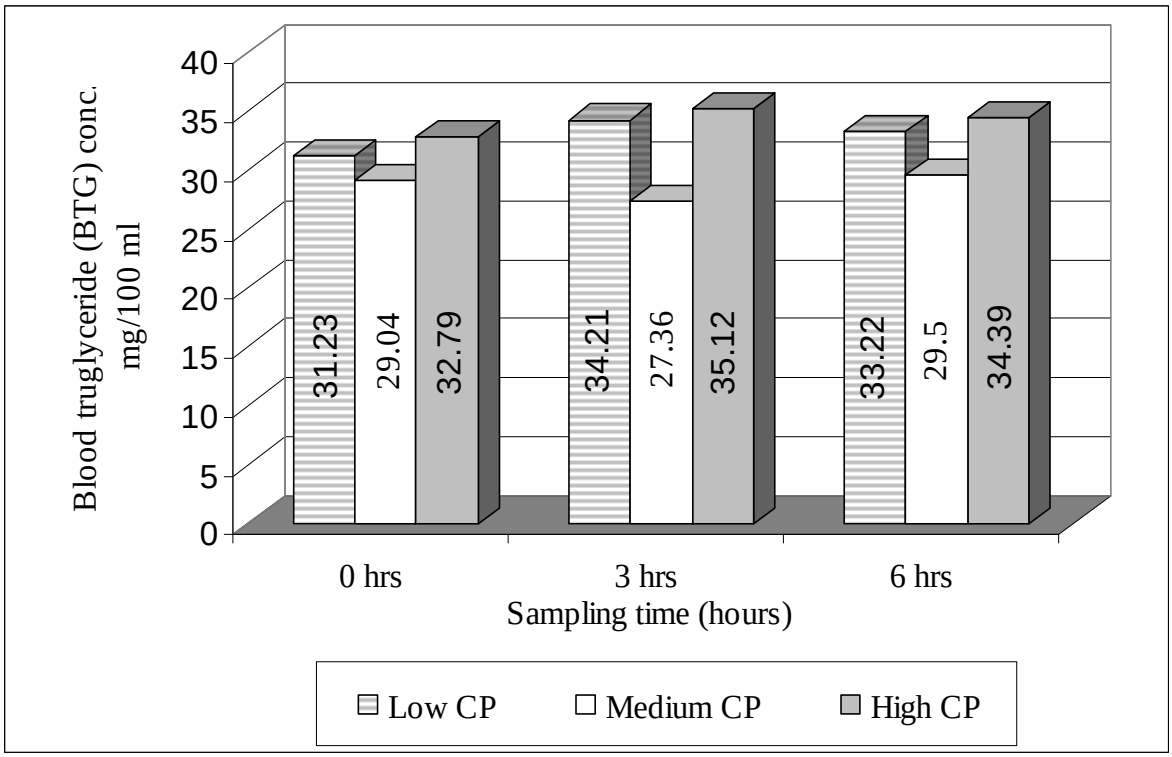

Figure 4- Diurnal pattern of blood triglyceride concentration (BTG) mg/100 ml

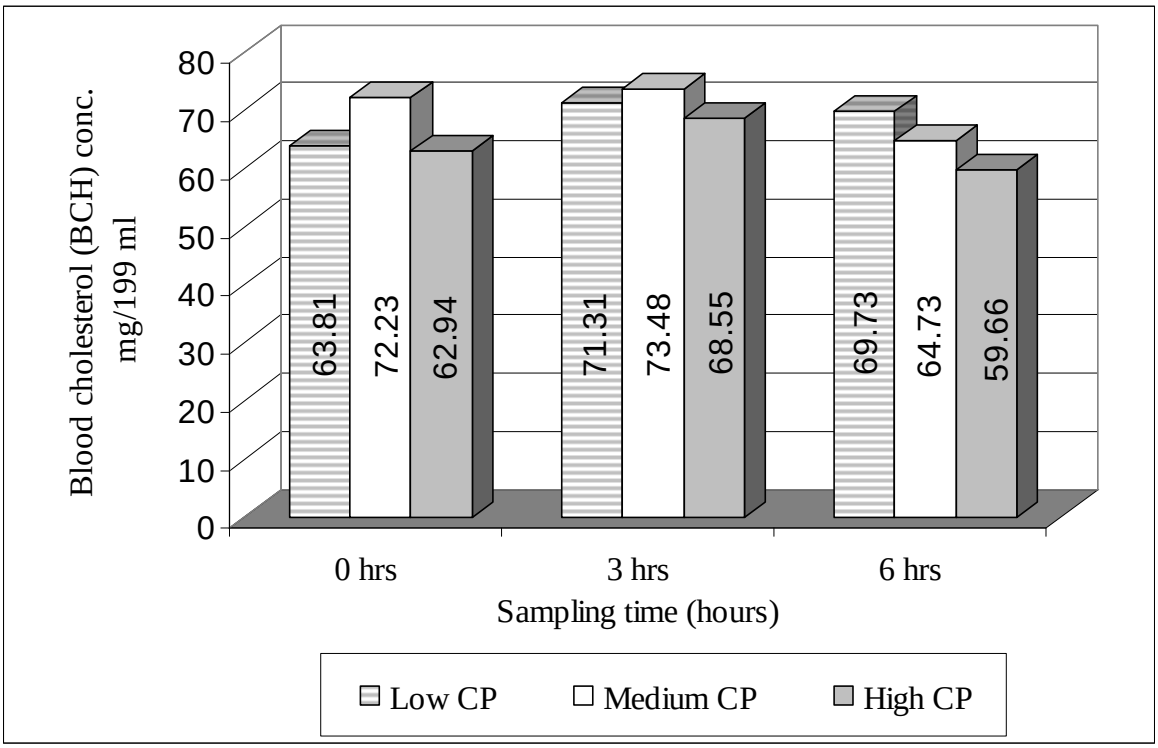

Figure 5- Diurnal pattern of blood cholesterol concentration (BCH) mg/100 ml

Table 5- Main effect of addition of SC (C) on blood parameters

	Addition of SC		Significance of effects
	Without	With	n = 72
BG mg.100 ml^{-1}	67.67^{b} ± 0.37	71.49^{a} ± 0.50	**
BTP g .100 ml^{-1}	6.55^{b} ± 0.06	7.06^{a} ± 0.11	*
BUN mg.100 ml^{-1}	44.88^{a} ± 0.81	41.01^{b} ± 0.90	**
BTG mg.100 ml^{-1}	31.39 ± 0.69	32.36 ± 0.80	NS
BCH mg.100 ml^{-1}	67.62 ± 1.30	67.14 ± 1.71	NS

Means having different letters at the same row are significantly different.

* (P<0.05) ** (P<0.01) NS= non significant

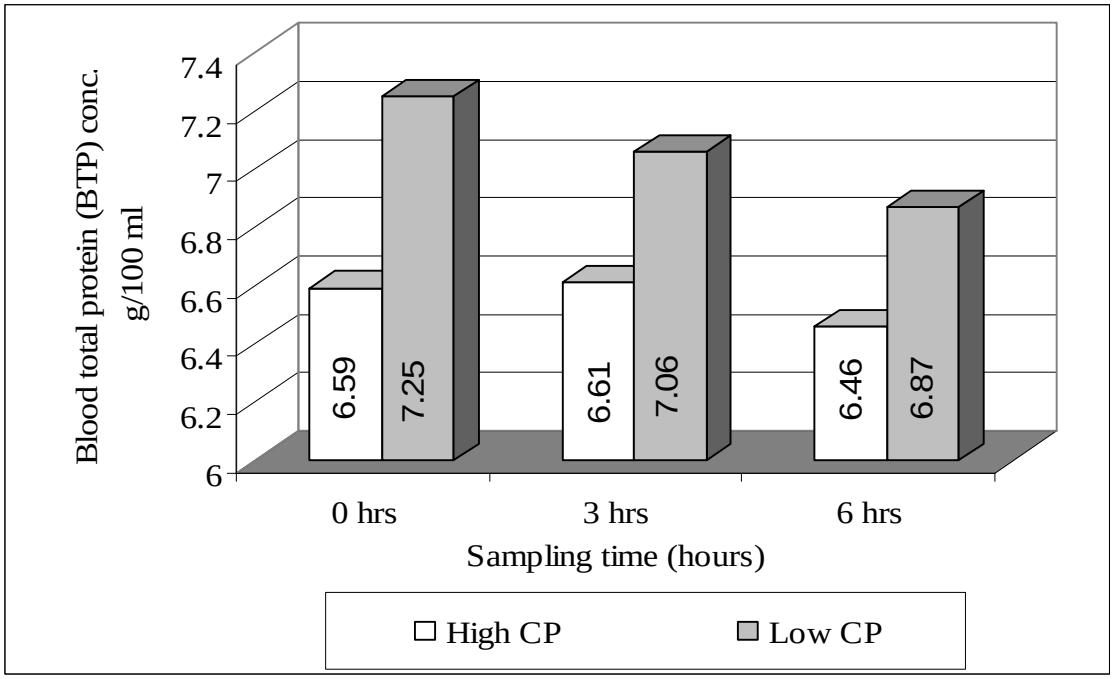

Figure 6- Diurnal pattern of blood glucose concentration (BG) mg/100 ml

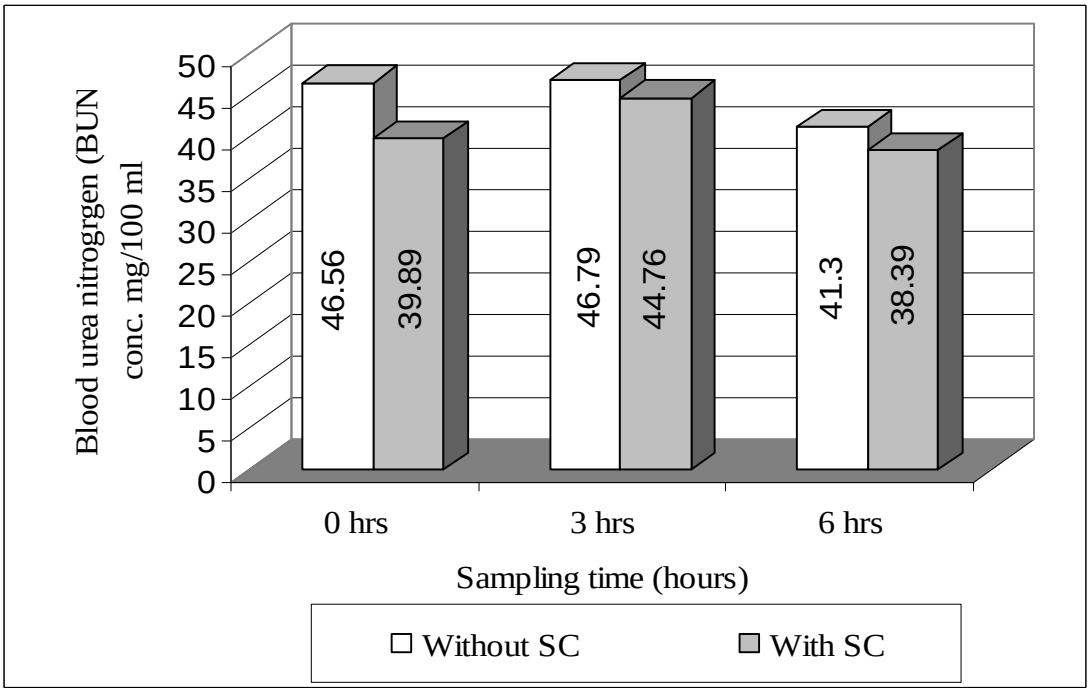

Figure 7- Diurnal pattern of blood total protein concentration (BTP) g/100 ml

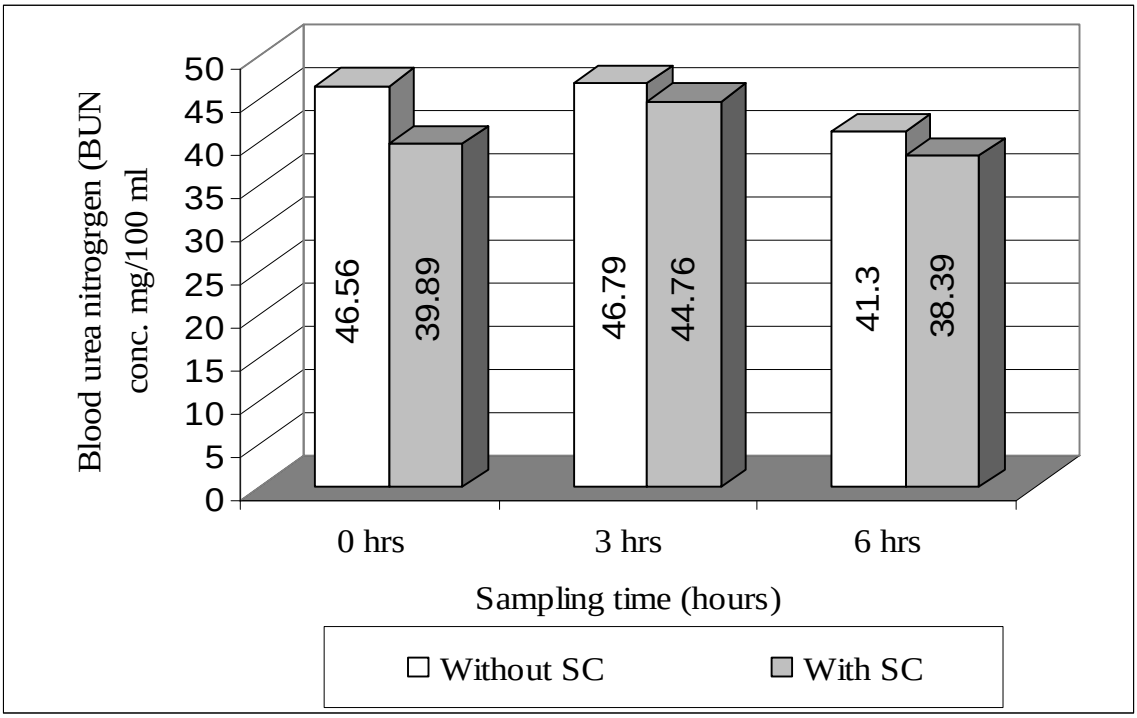

Figure 8- Diurnal pattern of blood urea nitrogen concentration (BUN) mg/100 ml

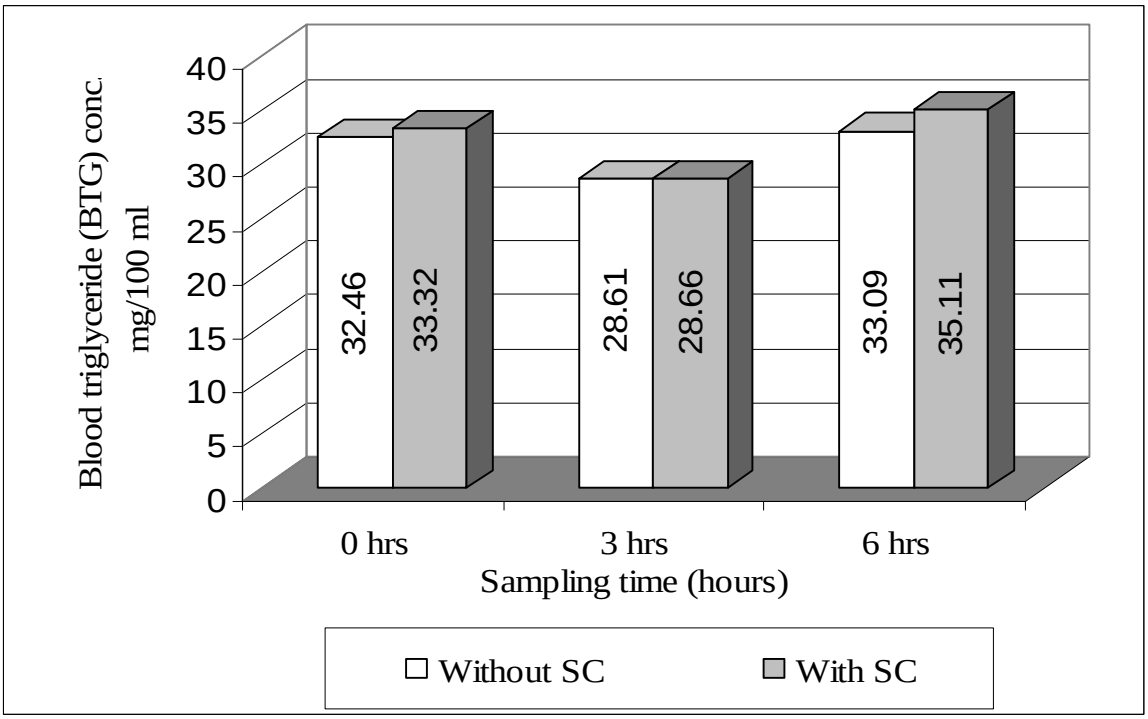

Figure 9- Diurnal pattern of blood triglyceride concentration (BTG) mg/100 ml

Table 6- Main effect of levels of dietary protein × addition of SC (A×C) on blood parameters

	Interactions						Significance of effect
	A_1C_1	A_1C_2	A_2C_1	A_2C_2	A_3C_1	A_3C_2	n = 24
BG mg.100 ml^{-1}	$68.77^{cd} \pm 0.58$	$73.07^{a} \pm 0.81$	$67.38^{d} \pm 0.71$	$71.53^{ab} \pm 0.70$	$66.88^{d} \pm 0.56$	$69.87^{bc} \pm 0.87$	*
BTP g .100 ml^{-1}	$6.50^{b} \pm 0.12$	$6.78^{ab} \pm 0.22$	$6.42^{b} \pm 0.09$	$7.21^{a} \pm 0.17$	$6.74^{b} \pm 0.08$	$7.19^{a} \pm 0.17$	*
BUN mg.100 ml^{-1}	$42.43^{bc} \pm 0.76$	$39.51^{c} \pm 1.08$	$43.95^{b} \pm 1.24$	$40.33^{bc} \pm 1.27$	$48.26^{a} \pm 1.61$	$43.20^{bc} \pm 2.06$	**
BTG mg.100 ml^{-1}	31.02 ± 1.05	31.02 ± 1.21	31.05 ± 1.43	33.41 ± 1.79	32.09 ± 1.17	32.65 ± 1.10	NS
BCH mg.100 ml^{-1}	$65.60^{bc} \pm 2.19$	$67.05^{abc} \pm 1.82$	$69.20^{ab} \pm 1.31$	$73.02^{a} \pm 2.93$	$68.06^{abc} \pm 3.03$	$61.35^{c} \pm 3.15$	*

Means having different letters at the same row are significantly different.

* ($P<0.05$) ** ($P<0.01$) NS= non significant.

A_1, A_2 and A_3 represent low, medium and high level of CP respectively, $C_1$and C_2 represent 0 and 0.5% of SC level respectively.

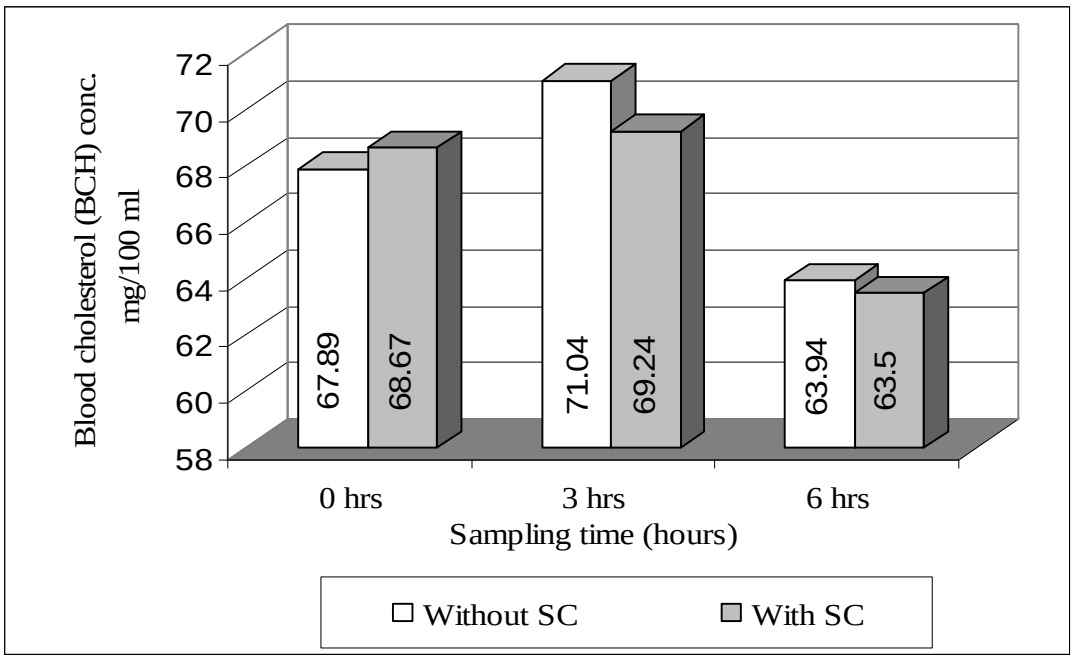

Figure 10- Diurnal pattern of blood cholesterol concentration (BCH) mg/100 ml

sheep when living cells of S. cerevisiae were added. They found 7-8 g more ruminal NAN pool in yeast-treated sheep in addition to 5 mg N were supplied through the yeast matter.

BUN concentration was decreased ($P<0.01$) due to addition of SC. This can be explained by the increase of utilization of ruminal ammonia by rumen microbes, because the BUN concentration was closely related to the ruminal NH_3-N evolutions (28). Physiologically, Milewski and Sobiech, (20) suggested that yeast supplementation had a protective effect on renal function as evidence from a decrease in BUN and creatinine concentrations.

Statistical analysis revealed that neither BTG nor BCH was significantly affected by addition of SC. Similar finding was obtained by Milewski and Sobiech (20). It was noted that the reserve fat in high producing ruminant was mobilized to compensate the negative energy balance which was often observed leading to a temporary rise in BTG (8). Since, BTG concentration was not affected in the current study due to addition of SC; there was probably a supported influence for yeast on

energy metabolism (20). Suskovic, et. al., (27) indicated that inclusion probiotics in ruminant diets reduced concentration of BCH; however, the mechanisms are still unknown. Diurnal changes in blood parameters as affected by addition of yeast are presented in Figures 6, 7, 8, 9 and 10. Main effect of the interactions between levels of dietary protein and addition of SC (A×C) on blood parameters

Results indicated that the effect of this interaction on blood glucose (BG) concentration was due to addition of SC since BG was not affected by increasing level of CP. Higher values due to addition of SC were observed within each level of dietary CP; the stimulated effect of yeast on BG was previously discussed. This increase of BG as affected by addition of SC may be attributed to the enhancement of propionogenesis process by yeast (19, 17).

Results also showed that higher BTP concentrations were detected due to the addition of SC, however, the significant ($P<0.05$) differences were limited to medium and high levels of CP; the positive effect of presence of yeast on BTP has been reported by Galip, (9).

With respect to blood urea nitrogen (BUN) concentrations, there was double response for the interaction between level of dietary CP and addition of SC because BUN concentration was significantly ($P<0.01$) influenced by both factors, though, their effects are inconsistent, where, as shown from table 4, increasing level of dietary CP increased ($P<0.01$) BUN, while it was decreased ($P<0.01$) due to addition of SC (table 5). As a result, higher values were detected in blood samples withdrawn from lambs fed high level of CP without addition of yeast as compared to BUN detected in samples withdrawn from lambs fed the low and medium levels of CP without addition of SC too. Correspondingly, higher BUN concentration was observed with the same pattern when SC was added.

With respect to blood triglyceride (BTG) results revealed that it was not affected, whereas, higher ($P<0.05$) cholesterol concentrations (BCH) was detected in blood sample withdrawn from lambs fed medium level of CP formulated with added SC. This inconsistent trend was caused by the factors which contributed to these interactions on both blood parameters

when their main effects were investigated separately.

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تاثير تغذية مستويات مختلفة من البروتين الغذائي واضافة خميرة الخبز (Saccharomyces cerevisiae) في اداء الحملان العواسية

3- معايير الدم

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المستخلص

استخدم 24 حملا عواسي لدراسة التغيرات الكيموحيوية في عناصر الدم بتاثير تقديم الأعلاف المركزة المحتوية على مستويات مختلفة من البروتين الخام الغذائي (واطىء ومتوسط ومرتفع) مع او بدون خميرة الخبز (Saccharomyces cerevisiae) (0 و 0.5 %). وقد قدمت الأعلاف المركزة الى الحملان بمعدل 3% من وزن الجسم بالأضافة الى كميات حرة من تبن الشعير. وقد سحبت نماذج الدم من الحملان قبل التغذية (وقت 0) و بعد التغذية بأوقات 3 و 6 ساعة. اظهرت النتائج بأن تركيز نتروحين يوريا الدم قد تاثر بالعاملين المذكورين و بصورة متعاكسة حيث ارتفع نتيجة لزيادة مستوى البروتين الخام وقد سجل اعلى ($P<0.01$) تركيز (45.73 ملغم . 100 مل$^{-1}$) عند تغذية المستوى المرتفع بالمقارنة مع المستوى الواطىء (40.97 ملغم . 100 مل$^{-1}$) ، بينما انخفض ($P<0.01$) ذلك التركيز من 44.88 الى 41.01 ملغم / 100 مل نتيجة لأضافة الخميرة. كما سجل اعلى تركيز لكلوكوز الدم ($P<0.01$) والبروتين الكلي في الدم ($P<0.05$) في نماذج الدم المسحوبة من الحملان التي اكمل غذائها بالخميرة. ولم يتأثر تركيز الكلوكوز والبروتين الكلي والكليسريدات الكلية نتيجة لزيادة مستوى البروتين الغذائي. كما ان تركيز عناصر الدم المرتبطة بالدهن (كوليستيرول الدم والكليسريدات الكلية) لم تتأثر باضافة الخميرة.

الكلمات المفتاحية : حملان العواسي ، البروتين الغذائي ، خميرة الخبز و معاييرالدم